

DNA CAPILLARY ELECTROPHORESIS

A. SCOPE

Amplified DNA fragments are separated by capillary electrophoresis (CE) on an AB 3130 Genetic Analyzer. Fluorescence detection is accomplished by utilizing an argon-ion multi-line, single mode laser with primary excitation lines at 488 nm and 514.5 nm to excite fluorescent dyes and a CCD camera that records the fluorescence emitted from samples within each of the four capillaries. The resulting data is subsequently analyzed using GeneMapper ID-X software. The fragment size is automatically assigned based on an internal size standard, which is co-electrophoresed with each sample. Alleles are assigned based on comparison of the fragment size of the unknown peak to that of the allelic ladder.

For the purpose of this manual, the following definitions apply:

Injection: all samples that are separated via CE at a single time. Thus for one injection 4 samples can be separated at a single time.

Run: all samples that were defined on a single plate and loaded onto the instrument at a single time for separation, regardless of the number of injections utilized to separate each sample contained on the plate. Thus, a run could contain up to 96 samples for each separation on a single plate.

B. QUALITY CONTROL

- B.1. Protective gloves and a lab coat must be worn when performing this procedure to prevent contamination.
- B.2. See DOC ID [1835](#) to determine reagent expiration dates.
- B.3. Do not clean any components or accessories of the 3130 with bleach or ethanol. Clean with deionized water.
- B.4. Hi-Di Formamide: To prevent repeated thawing and re-freezing of formamide, aliquot formamide into approximately 500 and 1000 µL volumes after initially thawing the 25 mL bottle. Appropriately discard any unused aliquot of thawed formamide.

C. SAFETY

- C.1. Hi-Di Formamide: exposure causes eye, skin, and respiratory tract irritation. It is also a possible developmental and birth defect hazard.
- C.2. All appropriate SDS sheets must be read prior to performing this procedure.
- C.3. Protective gloves, a lab coat and eye protection (e.g. safety glasses or a face shield) must be worn at all times when performing this procedure.

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D. REAGENTS, STANDARDS, AND CONTROLS

- D.1. GlobalFiler Allelic Ladder
- D.2. PowerPlex 16 HS Allelic Ladder
- D.3. Yfiler Allelic Ladder
- D.4. 3130 Performance Optimized Polymer (POP-4 polymer)
- D.5. AB 3130 Genetic Analyzer 10X Buffer w/ EDTA. To make a 1X working buffer:
 - D.5.1 Add 25 mL of 10X Buffer to 225 mL of deionized water to make 250 mL of working buffer, or make up a 1000 mL of the working buffer by adding 100 mL of 10X Buffer (4 bottles) to 900 mL deionized water.
- D.6. GS-600 LIZ Size Standard
- D.7. ILS-600 Size Standard
- D.8. GS-500 LIZ Size Standard
- D.9. Hi-Di Formamide

E. EQUIPMENT & SUPPLIES

- E.1. Equipment
 - E.1.1 AB 3130 Genetic Analyzer (instrument, computer and appropriate software)
 - E.1.2 AB 36cm capillary array
 - E.1.3 AB 3130 Genetic Analyzer sample septa and plates
 - E.1.4 Thermal cycler
 - E.1.5 Pipettes
 - E.1.6 Vortexer
 - E.1.7 Frozen plate block
 - E.1.8 Decapper
 - E.1.9 96-well plate retainer and base
 - E.1.10 96-well plate centrifuge
- E.2. Supplies
 - E.2.1 3130 Genetic Analyzer buffer vials/reservoirs/reservoir septa
 - E.2.2 Pipette tips
 - E.2.3 Microcentrifuge tubes
 - E.2.4 Strip caps
 - E.2.5 Parafilm
 - E.2.6 50 mL conical tubes
 - E.2.7 Scalpel
 - E.2.8 Permanent marker
 - E.2.9 Gloves
 - E.2.10 Lab coat

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- E.2.11 Eye protection (e.g. safety glasses, face shield)
E.2.12 [3130 template](#) (Save as .txt file before importing on 3130s)

F. PROCEDURES

F.1. GENERAL

- F.1.1 If the instrument is off, boot up the computer and then turn the instrument on. There are three indicator lights on the front of the instrument. Solid yellow indicates, “getting ready”, blinking yellow indicates the firmware is being sent, green light indicates “ready”, and the red light indicates “failure to connect, start over”.
- F.1.2 After a solid green light is indicated, launch the data collection software by clicking on “**Run 3130 data collection**” on the desktop.
- F.1.3 Four services (messaging, data, instrument and viewer) are launched automatically. When each has an indicator of a green box the program is ready.
- F.1.4 From the menu tree, open **ga3130**.
- F.1.5 Open “**3130**” to access the Wizards in the tool bar. The wizards are the following step by step self guided protocols:
- F.1.5.1. **Install Array Wizard:** This guides the operator through installation of a new or previously used 4 capillary array. This wizard includes a step to flush and fill the water seal trap. Select 36 cm for capillary length.
 - F.1.5.2. **Change Polymer Type Wizard:** This wizard is not utilized as all applications use the POP-4 polymer at this time.
 - F.1.5.3. **Replenish Polymer Wizard:** This wizard guides the operator through changing the polymer in the capillaries.
 - F.1.5.4. **Bubble Remove Wizard:** This wizard removes bubbles in the lines. This is necessary for proper electrophoresis.
 - F.1.5.5. **Instrument Shutdown Wizard:** This wizard guides the operator through the steps to remove the capillary array and shut down the instrument.
 - F.1.5.6. **Water Wash Wizard:** This wizard guides the operator through performing a wash of the polymer block, channels, and tubing with water.

Note: Stop the wizard after performing the water wash; ensure that the anode and cathode buffer reservoirs are filled with buffer and that the bottle connected to the polymer supply tube is filled with water.
 - F.1.5.7. **Autosampler Calibration Wizard:** This wizard guides the operator through calibrating the autosampler.
 - F.1.5.8. **Update Capillary Array Info:** This wizard guides the operator through the steps to update the capillary information.

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F.1.6 If a new capillary array needs to be installed on the instrument, a spatial calibration must be completed prior to running samples on the instrument. Proceed to DOC ID [1767](#) for instructions on performing a spatial calibration. A Yfiler spectral calibration, PowerPlex 16 HS spectral calibration and GlobalFiler spectral calibration may also be performed but are not required; proceed to DOC ID's [1768](#), [1769](#) and [12669](#) for instructions on performing a Yfiler spectral calibration, PowerPlex 16 HS spectral calibration and GlobalFiler spectral calibration, respectively.

F.1.7

F.1.8 Ensure adequate levels of 1X buffer are in the respective reservoirs (reservoir 1, as shown in Figure 1, and the anode buffer reservoir, as shown in Figure 3) and the outside of the reservoirs are dry. When replacing the 1X running buffer, the water reservoirs should also be rinsed and refilled with deionized water to the fill line. Figure 1 shows a diagram of the reservoirs, indicating which contain water and buffer.

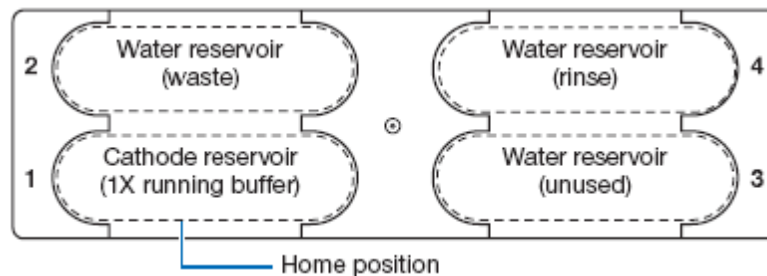
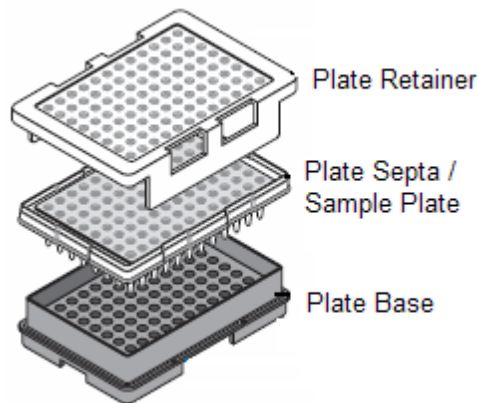


Figure 1 Location of buffer and water reservoirs on the 3130 (picture taken from AB 3130/3130xl Genetic Analyzers Maintenance, Troubleshooting, and Reference Guide)

F.1.9 Ensure the plate assemblies are properly assembled (as shown in Figure 2) and positioned on the deck properly. Note: if the plate assembly is properly assembled, the gray septa will not be visible when looking down through the holes of the plate retainer. Instead, the wells will appear black. If the plate assembly is not properly assembled, damage to the capillary array is likely to occur.



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Figure 2 Plate assembly (picture taken from AB 3130/3130xl Genetic Analyzers Maintenance, Troubleshooting, and Reference Guide)

- F.1.10 Check for bubbles in the pump block, lower polymer block, interconnect tube, polymer supply tube, and channels. If bubbles are present, remove all bubbles with the Bubble Remove wizard.
- F.1.11 Ensure the capillary tips are not crushed or damaged.
- F.1.12 Ensure that sufficient volume of polymer is present in the bottle for a run. (Note: approximately 0.5 mL of polymer is sufficient to run approximately a plate of samples without introduction of bubbles into the pump block. Additional polymer may be necessary to clear bubbles, etc.)
- F.1.13 Ensure that the pump block and lower polymer block are securely positioned on the instrument.
- F.1.14 Ensure that the instrument surfaces are clean; always use deionized water to clean.
- F.1.15 Check for leaks around the array knob, interconnecting tube nuts, and check valve, all of which are shown in Figure 3.

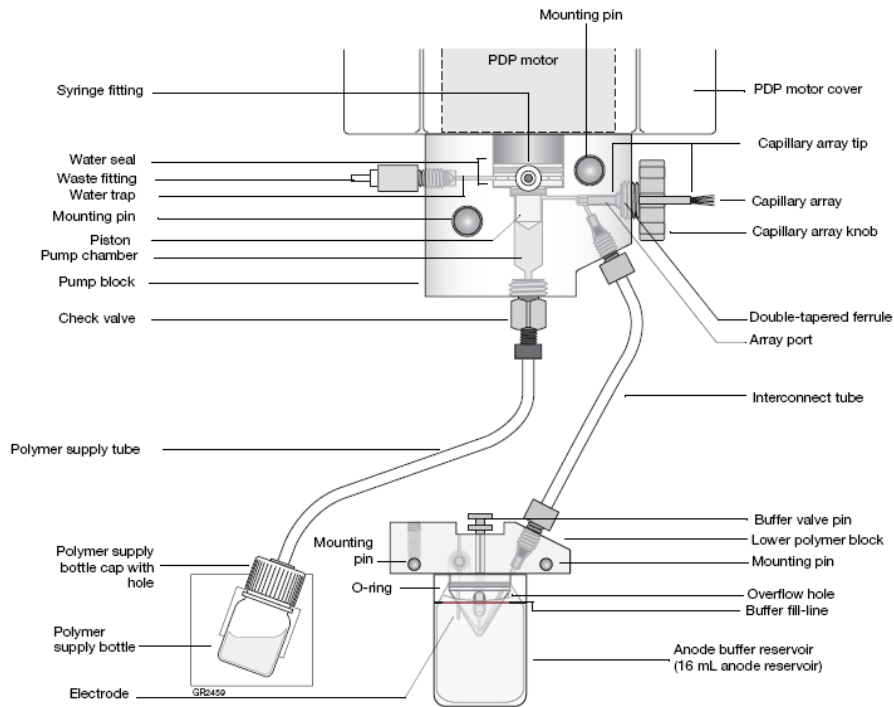


Figure 3 Diagram of the pump block and associated components of the AB 3130 Genetic Analyzer (Picture taken from AB 3130/3130xl Genetic Analyzers Maintenance, Troubleshooting, and Reference Guide).

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F.2. FLUSHING AND FILLING THE WATER TRAP

The water trap should be flushed with either distilled or deionized water as needed to wash any diluted polymer and to clear bubbles. Leave the trap filled with either distilled or deionized water.

- F.2.1 Fill the 20 mL all-plastic Luer lock syringe with distilled or deionized water. Expel any bubbles from the syringe. Do not use a smaller syringe as doing so may generate excessive pressure within the trap.
- F.2.2 Attach the syringe to the forward facing Luer fitting at the top of the pump block. Hold the fitting with one hand while threading the syringe onto the fitting with the other hand.
- F.2.3 Open the Luer fitting by grasping the body of the fitting and turning it and the attached syringe approximately one-half turn counterclockwise.
- F.2.4 Open the exit fitting at the top left side of the pump block by turning it approximately one-half counterclockwise.
- F.2.5 Hold an empty tube or beaker under the exit fitting to receive approximately 5 mL of water. Flush the trap by pushing steadily on the syringe. **DO NOT USE EXCESSIVE FORCE** when you push the syringe plunger as this may damage the trap seals.
- F.2.6 Close the fittings in the following order by turning each clockwise until the fittings seal against the block:
 - Luer fitting
 - Exit fitting
 - Remove the syringe from the Luer fitting. Hold the fitting with one hand while turning the syringe counterclockwise with the other hand.

NOTE: Do not over-tighten the fittings as excessive tightening can damage the fittings. Very little pressure develops within the trap during pump operation, so fittings require only enough tightening to prevent water leaks,

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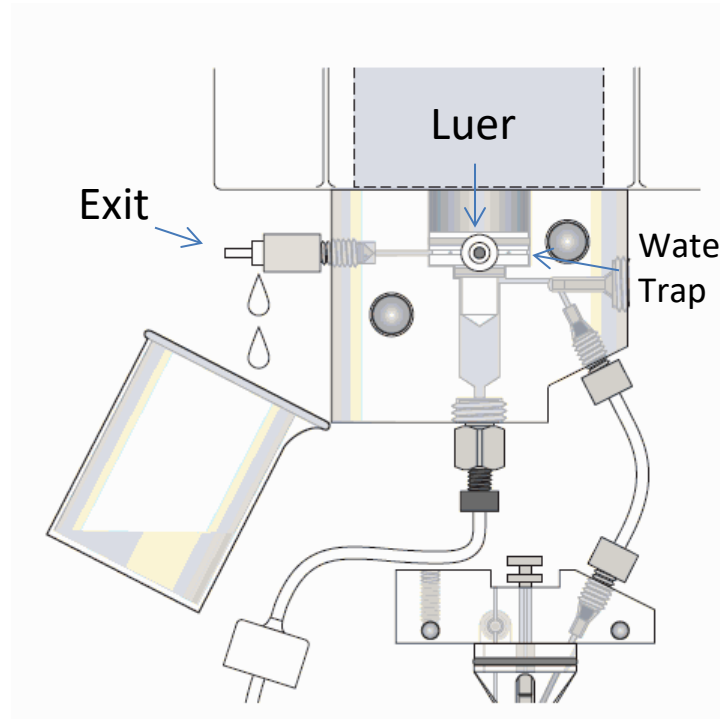


Diagram of the water trap and associated components of the AB 3130 Genetic Analyzer (Picture taken from AB 3130/3130xl Genetic Analyzers Maintenance, Troubleshooting, and Reference Guide. Names added).

F.3. CREATING A PLATE DOCUMENT

- F.3.1 Under **ga3130** open **plate manager**, select **new**, Fill in the plate name. Select **GeneMapper-Generic** for the application. Select **96-well** for plate type. Owner and Operator name are required fields. The analyst can put their initials in these fields. Select **OK** when the entry fields are complete.
- F.3.2 A GeneMapper plate editor appears on the screen. Fill in the sample name column with the sample name that corresponds to the plate position. No spaces, /, \, :, *, ", <, >, and ? may be in the sample name.
- F.3.3 OPTIONAL: Add the appropriate sample type e.g. sample, allelic ladder, positive control or negative control.
- F.3.4 OPTIONAL: Add **CE_G5_HID_GS500**, **ILS-600** or **GS600_LIZ_(60-460)** for the size standard for all samples amplified with Yfiler, PowerPlex 16 HS or GlobalFiler, respectively.
- F.3.5 OPTIONAL: Add the **Yfiler**, **PowerPlex** or **GlobalFiler** panel.
- F.3.6 OPTIONAL: Add the **Yfiler**, **PowerPlex** or **GlobalFiler** analysis method.

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- F.3.7 Select **Identifiler_NoAutoAnalysis** in the results group. To create a new results group refer to DOC ID [6235](#).
- F.3.8 Select **Identifiler_3sec**, **Identifiler_5sec** or **Identifiler_8sec** for samples amplified with Y filer or **PP16HS_3sec**, **PP16HS_5sec** or **PP16HS_8sec** for samples amplified with PowerPlex16 HS or **Globalfiler-3sec**, **Globalfiler-5sec** or **Globalfiler-8sec** for samples amplified with GlobalFiler under Instrument protocol 1. **Note:** names may vary between instruments. To create a new protocol, refer to DOC ID [6222](#).
- F.3.9 Additional injections of the same sample can be created by selecting **Edit/Add sample run** or Alt+A. When this is selected an additional Results Group and Instrument Protocol column are added to the GeneMapper Plate Editor. Select the current Results Group e.g. **Identifiler_NoAutoAnalysis**. Select the desired Instrument protocol for a varied injection time e.g. 3, 5 or 8 sec injections for all kits.
- F.3.10 OPTIONAL: To analyze samples in the order set up on the plate, fill the comments column in numerical order. Numbers 1 -9 must be entered as 01, 02, 03 etc.
- F.3.11 Set up the plate with the samples in the appropriate position.
- F.3.12 When the plate setup is complete select **OK**.

F.4. IMPORTING A PLATE DOCUMENT

A 3130 plate template may also be utilized to create a plate document; this document can then be imported using the plate manager. The file will not import if any illegal characters are present throughout the document. These include: spaces, /, \, :, *, ", <, >, and ? in the file name or sample names. In addition unused wells must be deleted prior to file import and the plate must have a unique name in cell A2 of the file.

- F.4.1 Under ga3130 open plate manager select import file. Highlight the file that you would like to import and press the **Import** button.

F.5. 3130 RUN SCHEDULER

- F.5.1 Open the **ga3130/3130/Run Scheduler** in data collection.
- F.5.2 Select search (with criteria in desired fields) or **Find All** to locate the desired plate document.
- F.5.3 Highlight the desired plate document by clicking on it.
- F.5.4 Click on the yellow plate to link the plate document to the plate that is in the 3130 instrument. When the plate document is successfully linked the plate picture turns green.
- F.5.5 Click the green arrow in the upper left corner to start the run.
- F.5.6 A message box appears "You are about to start processing plates..." select **OK**.

F.6. YFILER SAMPLE PREPARATION FOR CE

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- F.6.1 Import or prepare plate document as described in sections F.3 and F.4.
- F.6.2 Prepare the 96 well sample plate by placing into a plate base and labeling appropriately.
- F.6.3 Combine the necessary amount of formamide and GeneScan-500 [LIZ] size standard in a single microcentrifuge tube as follows:

(Number of samples + 2) x 24.5 µL formamide

(Number of samples + 2) x 0.5 µL GeneScan-500 [LIZ] size standard

Note: *It is recommended that enough volume for additional samples be included in the calculation to account for volume lost in pipetting; therefore, depending on the number of samples, more than two extra samples can be incorporated into the above listed calculation.*

- F.6.4 Vortex and spin the mixture briefly in a microcentrifuge.
- F.6.5 Aliquot 25 µL of the formamide / GeneScan-500 LIZ master mix into each plate well.
- F.6.6 Add 1.5 µL of PCR product or allelic ladder to each plate well. Cover the plate with a rubber septa.
- F.6.7 Centrifuge the plate briefly.
- F.6.8 Heat the samples in a thermal cycler for three minutes at 95°C to denature.
- F.6.9 Snap-cool immediately for a minimum of three minutes in frozen plate holder.
- F.6.10 Place the plate into the plate base and centrifuge briefly.
- F.6.11 Secure sample plate into the base with a plastic retainer clip (Figure 2).
- F.6.12 Place tray onto autosampler with well A1 position at the back right hand corner.
- F.6.13 Link plate to run by clicking on the yellow plate diagram. Select **green arrow** from the **run scheduler** window to start the run (See F.5).
- F.6.14 When the run is complete, a copy of the raw data will be saved in the analyst's casework folder on the "I" drive. The analyst should maintain case folders in monthly files. The data from the first analysis of any convicted offender sample should be stored under K:\Division\DNA\CODIS\Analysis.

F.7. POWERPLEX 16 HS SAMPLE PREPARATION FOR CE

- F.7.1 Import or prepare plate document as described in section F.3 and F.4.
- F.7.2 Prepare the 96 well sample plate by placing into a plate base and labeling appropriately.
- F.7.3 Combine the necessary amount of formamide and ILS 600 size standard in a single microcentrifuge tube as follows:

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(Number of samples + 2) x 24 µL formamide

(Number of samples + 2) x 1 µL ILS 600 size standard

Note: *It is recommended that enough volume for additional samples be included in the calculation to account for volume lost in pipetting; therefore, depending on the number of samples, more than two extra samples can be incorporated into the above listed calculation.*

- F.7.4 Vortex and spin the mixture briefly in a microcentrifuge.
- F.7.5 Aliquot 25 µL of the formamide / ILS 600 master mix into each plate well.
- F.7.6 Add 1 µL of PCR product or allelic ladder to each plate well. Cover the plate with a rubber septa.
- F.7.7 Centrifuge the plate briefly.
- F.7.8 Heat the samples in a thermal cycler for three minutes at 95°C to denature.
- F.7.9 Snap-cool immediately for a minimum of three minutes in frozen plate holder.
- F.7.10 Place the plate into the plate base and centrifuge briefly.
- F.7.11 Secure sample plate into the base with a plastic retainer clip (Figure 2).
- F.7.12 Place the tray onto the autosampler with position A1 at the back right.
- F.7.13 Link plate to run by clicking on the yellow plate diagram. Select **green arrow** from the **run scheduler** window to start the run (See F.5.).
- F.7.14 When the run is complete, a copy of the raw data should be saved in the analyst's casework folder on the "I" drive. The analyst should maintain case folders in monthly files. The data from the first analysis of any convicted offender sample should be stored under K:\Division\DNA\CODIS\Analysis.

F.8. GLOBALFILER SAMPLE PREPARATION FOR CE

- F.8.1 Import or prepare plate document as described in section F.3 and F.4.
- F.8.2 Prepare a 96 well sample plate by placing it into a plate base and labeling it appropriately.
- F.8.3 Combine the necessary amount of formamide and GS 600 LIZ size standard in a microcentrifuge tube as follows:

(Number of samples + 2) x 19.2 µL formamide

(Number of samples + 2) x 0.8 µL GS 600 LIZ

Note: *It is recommended that enough volume for additional samples be included in the calculation to account for volume lost in pipetting; therefore, depending on the number of samples, more than two extra samples can be incorporated into the above listed calculation.*

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- F.8.4 Vortex and spin the mixture briefly in a microcentrifuge.
- F.8.5 Aliquot 20 µL of the formamide/GS 600 LIZ master mix into each plate well.
- F.8.6 Add 2 µL of PCR product or allelic ladder to each plate well. Cover the plate with rubber septa.
- F.8.7 Centrifuge the plate briefly.
- F.8.8 Heat the samples in a thermal cycler for three minutes at 95°C to denature.
- F.8.9 Snap-cool immediately for a minimum of three minutes in frozen plate holder.
- F.8.10 Place the plate into the plate base and centrifuge briefly.
- F.8.11 Secure sample plate into the base with a plastic retainer clip (Figure 2).
- F.8.12 Place the tray onto the autosampler with position A1 at the back right.
- F.8.13 Link plate to run by clicking on the yellow plate diagram. Select **green arrow** from the **run scheduler** window to start the run (See F.5.).
- F.8.14 When the run is complete, a copy of the raw data should be saved in the analyst's casework folder on the "I" drive. The analyst should maintain case folders in monthly files. The data from the first analysis of any convicted offender sample should be stored under K:\Division\DNA\CODIS\Analysis.

G. INTERPRETATION GUIDELINES

- G.1. See DOC ID [1773](#) (PP16HS interpretation guidelines), [1776](#) (Yfiler interpretation guidelines) and [12628](#) (GlobalFiler interpretation guidelines).

H. REFERENCES

- H.1. Applied Biosystems 3130/3130xl Genetic Analyzers Using Data Collection Software v3.0, February 8, 2005.
- H.2. Applied Biosystems 3130/3130xl Genetic Analyzers Getting Started Guide November 2004.
- H.3. Applied Biosystems 3130/3130xl Genetic Analyzers Maintenance, Troubleshooting, and Reference Guide September 2006.

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